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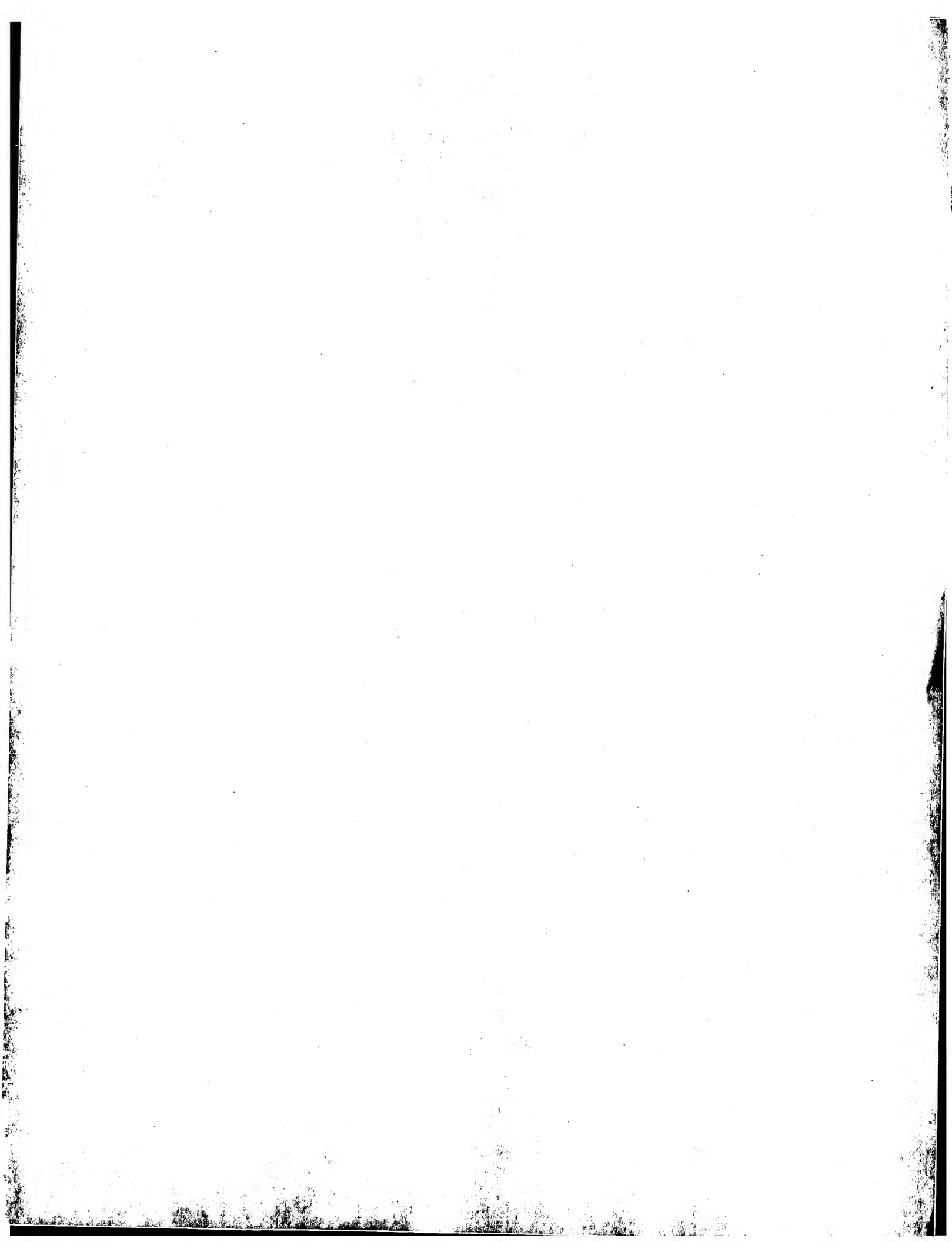
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02-24-04

PATENT  
674519-2029

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s) : Packham et al.  
U.S. Serial No. : 10/728,383  
Filing Date : December 5, 2003  
For : **COMPOSITIONS COMPRISING OESTRONE-3-0-  
SULPHAMATE AND TRAIL (TNF-RELATED  
APOPTOSIS INDUCING LIGAND)**

745 Fifth Avenue  
New York, NY 10151

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**CLAIM OF PRIORITY**

Sir:

Applicants hereby claim priority under 35 U.S.C. §119 and/or 120, from U.K Application Nos. 0113920.3 and International patent application number PCT/GB02/02541, a certified copy of each is enclosed.

Acknowledgment of the claim of priority and of the receipt of said certified copies is respectfully requested.

Respectfully submitted,

FROMMER LAWRENCE & HAUG LLP

By: Thomas J. Kowalski

THOMAS J. KOWALSKI, Reg. No. 32,147  
Tel. No. (212) 588-0800





INVESTOR IN PEOPLE

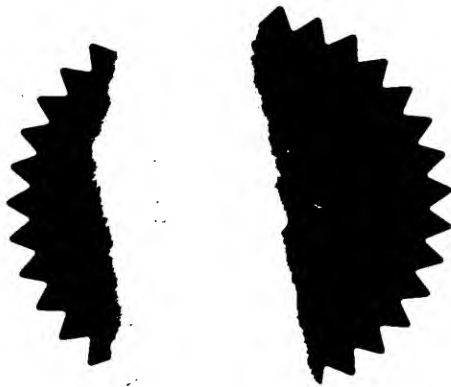
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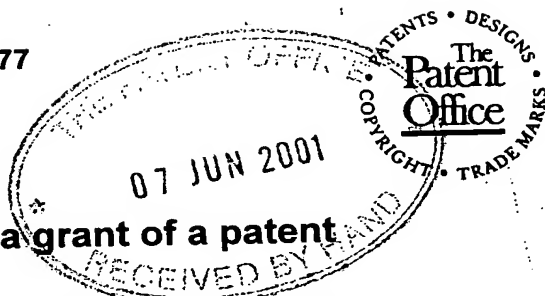




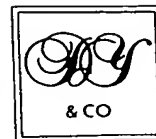
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1/77



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P010941GB DAA

08JUN01 E635589-4 D02246

P01/7700 0.00-0113920.3

2. Patent application number  
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07 JUN 2001

0113920.3

3. Full name, address and postcode of the  
or of each applicant  
(underline all surnames)

Sterix Limited  
Magdalen Centre  
Robert Robinson Avenue  
The Oxford Science Park  
Oxford  
OX4 4GA

Patents ADP number (if you know it)

07652134001

If the applicant is a corporate body, give  
the country/state of its incorporation

United Kingdom

4. Title of the invention

Composition

5. Name of your agent (if you have one)

D Young & Co

"Address for service" in the United Kingdom  
to which all correspondence should be sent  
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21 New Fetter Lane  
London  
EC4A 1DA

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59006

6. If you are declaring priority from  
one or more earlier patent  
applications, give the country and  
date of filing of the or each of these  
earlier applications and (if you know  
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1st

2nd

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Number of earlier  
application

Date of filing  
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a) any applicant named in part 3 is not an inventor, or  
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	Translations of Priority Documents	0
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	Request for preliminary examination and search (Patents Form 9/77)	0
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	Any other documents (Please specify)	0

11. I/We request the grant of a Patent on the basis of this application.

Signature

Date

*D Young & Co*  
**D YOUNG & CO**  
Agents for the Applicants

7 Jun 2001

12. Name and daytime telephone number of person to contact in the United Kingdom	David Alcock	023 8071 9500
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# COMPOSITION

The present invention relates to a composition. In particular the present invention relates to a pharmaceutical composition and to a use thereof.

5

Cancer remains a major cause of mortality in most Western countries. So far, evidence suggests that oestrogens are the major mitogens involved in promoting the growth of tumours in endocrine-dependent tissues, such as the breast and endometrium. Although plasma oestrogen concentrations are similar in women with or without breast cancer, breast  
10 tumour oestrone and oestradiol levels are significantly higher than in normal breast tissue or blood. *In situ* synthesis of oestrogen is thought to make an important contribution to the high levels of oestrogens in tumours and therefore specific inhibitors of oestrogen biosynthesis are of potential value for the treatment of endocrine-dependent tumours.

15 Over the past two decades, there has been considerable interest in the development of inhibitors of the aromatase pathway which converts the androgen precursor androstenedione to oestrone. However, there is now evidence that the oestrone sulphatase ("E1-STS") pathway, i.e. the hydrolysis of oestrone sulphate ("E1S") to oestrone ("E1"), as opposed to the aromatase pathway, is the major source of oestrogen in breast tumours.  
20 This theory is supported by a modest reduction of plasma oestrogen concentration in postmenopausal women with breast cancer treated by aromatase inhibitors, such as aminoglutethimide and 4-hydroxyandrostenedione and also by the fact that plasma E1S concentration in these aromatase inhibitor-treated patients remains relatively high. The long half-life of E1S in blood (10-12 h) compared with the unconjugated oestrogens (20  
25 min) and high levels of steroid sulphatase activity in liver and, normal and malignant breast tissues, also lend support to this theory.

Singh *et al* (1997 J Steroid Biochem Mol Biol 61: 185-192), report that the major source of pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6 within breast tumours is not well  
30 understood but it is thought that tumour infiltrating macrophages and lymphocytes might play a role.

Singh *et al (ibid)* also report that both TNF- $\alpha$  and IL-6 inhibit the growth of MCF-7 breast cancer cells *in vitro*. In addition, TNF- $\alpha$  has an inhibitory effect on aromatase activity  
35 measured in cultured MCF-7 breast cancer cells. Apparently, these results contrast with

the marked stimulatory effect that TNF- $\alpha$  has on fibroblasts derived from normal and malignant breast tissues (Macdiarmaid *et al* 1994 Molec. Cell Endoc. 106: 17-21). In addition, when TNF- $\alpha$  is combined with IL-6, the inhibitory effect on aromatase activity is enhanced. The synergistic inhibitory effect of IL-6 and TNF- $\alpha$  on aromatase activity in MCF-7 cells also contrasts to the synergistic stimulatory effect that these cytokines have on oestrone sulphatase and oestradiol dehydrogenase activities in these cells.

Tumour necrosis factor (TNF) related apoptosis inducing ligand (TRAIL/Apo-2L) induces apoptosis in a wide range of tumour cells by binding to the receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). These are members of the TNF $\alpha$  superfamily of death receptors, characterised by the possession of intracellular 'death domains', that are responsible for transduction of the death signal (Ashkenazi and Dixit 1998).

Treatment with TRAIL in combination with DNA damaging agents such as doxorubicin, etoposide and ionising radiation has been shown to significantly enhance the antitumour actions of TRAIL in breast cancer cells (Keane *et al.*, 1999, Gibson *et al.*, 2000, Chinnalyan *et al.*, 2000). The mechanism for this co-operation is thought to be due to the ability of DNA damaging agents to increase levels of DR5 and/or DR4 receptors, possibly mediated through upregulation of the tumour suppressor p53 or the transcription factor NF $\kappa$ B. (Wu *et al.*, 1997, Gibson *et al.*, 2000).

The present invention seeks to provide a composition suitable for use in the treatment of cancers and, especially, breast cancer.

According to a first aspect of the present invention there is provided a composition comprising (i) a compound comprising a sulphamate group ("a sulphamate compound"); and (ii) an apoptosis inducer.

According to a second aspect of the present invention there is provided a composition of the present invention for use in medicine.

According to a third aspect of the present invention there is provided a use of a composition of the present invention in the manufacture of a medicament to prevent and/or inhibit tumour growth.

According to a fourth aspect of the present invention there is provided a use of a composition of the present invention a composition of the present invention in the manufacture of a medicament to induce apoptosis.

- 5 According to a fifth aspect of the present invention there is provided a use of a sulphamate compound in the manufacture of a medicament to upregulate a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor.

According to a sixth aspect of the present invention there is provided a method of  
10 treatment comprising administering to a subject in need of treatment a composition of the present invention.

According to a seventh aspect of the present invention there is provided a method of treatment comprising administering to a subject in need of treatment a composition of the  
15 present invention or an sulphamate compound in order to induce apoptosis.

According to an eighth aspect of the present invention there is provided a kit comprising a part i) containing a compound comprising a sulphamate group ("a sulphamate compound"); and a part ii) containing an apoptosis inducer. The parts of the kit may be  
20 independently held in one or more containers - such as bottles, syringes, plates, wells, blister pack etc.

The present invention is advantageous in that it provides a composition suitable for use in the treatment of cancers and, especially, breast cancer.

25 In addition, the present invention is advantageous in that it provides a compound that is suitable for use in the treatment of cancers such as breast cancer, ovarian cancer, endometrial cancer, sarcomas, melanomas, prostate cancer etc. - especially, breast cancer.

30 Another advantage of the compositions of the present invention is that they may be more potent *in vivo* than the sulphamate compounds alone or the apoptosis inducer alone. Moreover, in some aspects the combination of sulphamate compounds and the apoptosis inducer is more potent than one would expect from the potency of the compound alone i.e.  
35 this is a synergistic relationship between them.

The synergistic combination of sulphamate compound and apoptosis inducer allows for the use of lower doses of either or both of the sulphamate compound and apoptosis inducer. This is particularly advantageous when therapeutic amounts required in non-synergistic systems are to be avoided, for example for reasons of toxicity of one of the components.

In accordance with the present invention the composition of the present invention may comprise more than one apoptosis inducer.

10 Preferably, the apoptosis inducer is an apoptosis inducing ligand.

Preferably, the apoptosis inducer is an apoptosis inducing cytokine.

A cytokine is a molecule - often a soluble protein - that allows immune cells to communicate with each other. These molecules exert their biological functions through specific receptors expressed on the surface of target cells. Binding of the receptors triggers the release of a cascade of biochemical signals which profoundly affect the behaviour of the cell bearing the receptor (Poole, S 1995 TibTech 13: 81-82). Many cytokines and their receptors have been identified at the molecular level (Paul and Sedar 1994, Cell 76: 241-251) and make suitable molecules of therapeutic value as well as therapeutic targets in their own right.

More details on cytokines can be found in Molecular Biology and Biotechnology (Pub. VCH, Ed. Meyers, 1995, pages 202, 203, 394, 390, 475, 790).

25

More preferably the cytokine is a tumour necrosis factor apoptosis inducing ligand (TRAIL). Yet more preferably the TRAIL is TRAIL/Apo-2L.

With this aspect of the present invention the compositions of the present invention are more potent *in vivo* than the sulphamate compounds alone or TRAIL alone. Moreover, in some aspects the combination of sulphamate compounds and TRAIL is more potent than one would expect from the potency of the compound alone i.e. this is a synergistic relationship between them.

35 The TRAIL can be prepared chemically or it can be extracted from sources. Preferably,

the TRAIL is prepared by use of recombinant DNA techniques.

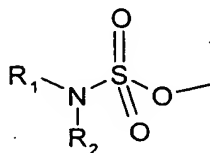
In a preferred aspect the apoptosis inducer is capable of interacting with a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor. Preferably the receptor is DR4 and/or DR5.

In accordance with the present invention the composition of the present invention may comprise more than one sulphamate compound.

The term "sulphamate compound" means a compound comprising at least one sulphamate group.

The term "sulphamate" includes an ester of sulphamic acid, or an ester of an N-substituted derivative of sulphamic acid, or a salt thereof.

Preferably, the sulphamate group of the sulphamate compound has the formula:



wherein each of R<sub>1</sub> and R<sub>2</sub> is independently selected from H or a hydrocarbyl group.

The term "hydrocarbyl group" as used herein means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, a hydrocarbon group, an N-acyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen.

In one preferred embodiment of the present invention, the hydrocarbyl group is a hydrocarbon group.

Here the term "hydrocarbon" means any one of an alkyl group, an alkenyl group, an alkynyl group, an acyl group, which groups may be linear, branched or cyclic, or an aryl group. The term hydrocarbon also includes those groups but wherein they have been optionally substituted. If the hydrocarbon is a branched structure having substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

Preferably,  $R_1$  and  $R_2$  are independently selected from H or alkyl, cycloalkyl, alkenyl and aryl, or together represent alkylene, wherein the or each alkyl or cycloalkyl or alkenyl or optionally contain one or more hetero atoms or groups.

When substituted, the N-substituted compounds of this invention may contain one or two N-alkyl, N-alkenyl, N-cycloalkyl, N-acyl, or N-aryl substituents, preferably containing or each containing a maximum of 10 carbon atoms. When  $R_1$  and/or  $R_2$  is alkyl, the preferred values are those where  $R_1$  and  $R_2$  are each independently selected from lower alkyl groups containing from 1 to 5 carbon atoms, that is to say methyl, ethyl, propyl etc. Preferably  $R_1$  and  $R_2$  are both methyl. When  $R_1$  and/or  $R_2$  is aryl, typical values are phenyl and tolyl ( $-\text{PhCH}_3$ ; *o*-, *m*- or *p*-). Where  $R_1$  and  $R_2$  represent cycloalkyl, typical values are cyclopropyl, cyclopentyl, cyclohexyl etc. When joined together  $R_1$  and  $R_2$  typically represent an alkylene group providing a chain of 4 to 6 carbon atoms, optionally interrupted by one or more hetero atoms or groups, e.g. -O- or -NH- to provide a 5-, 6- or 7-membered heterocycle, e.g. morpholino, pyrrolidino or piperidino.

Within the values alkyl, cycloalkyl, alkenyl, acyl and aryl we include substituted groups containing as substituents therein one or more groups which do not interfere with the sulphatase inhibitory activity of the compound in question. Exemplary non-interfering substituents include hydroxy, amino, halo, alkoxy, alkyl and aryl. A non-limiting example of a hydrocarbyl group is an acyl group.

In some preferred embodiments, at least one of  $R_1$  and  $R_2$  is H.

Preferably the sulphamate compound is a cyclic compound. In this regard, the sulphamate compound can be a single ring compound or a polycyclic compound. Here, the term "polycyclic" includes fused and non-fused ring structures including combinations

thereof.

Thus, preferably the sulphamate compound is of the formula

E-G

5 wherein E is a sulphamate group and wherein G is a cyclic group.

The cyclic group may be a single ring or it is a polycyclic ring structure.

10 In one aspect, the cyclic group may contain any one or more of C, H, O, N, P, halogen (including Cl, Br and I), S and P.

At least one of the cyclic groups may be a heterocyclic group (a heterocycle) or a non-heterocyclic group.

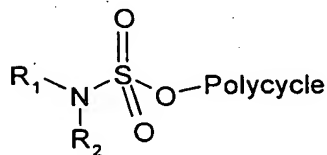
15 At least one of the cyclic groups may be a saturated ring structure or an unsaturated ring structure (such as an aryl group).

Preferably, at least one of the cyclic groups is an aryl ring.

20 Preferably, the sulphamate group is linked to the aryl ring.

If the cyclic group is polycyclic some or all of the ring components of the sulphamate compound may be fused together or joined *via* one or more suitable spacer groups.

25 Thus, in accordance with one aspect of the present invention, preferably the sulphamate compound is a polycyclic compound. In this aspect the sulphamate compound may be a compound having the formula



wherein each of R<sub>1</sub> and R<sub>2</sub> is independently selected from H or a hydrocarbyl group.

30

Preferably the polycyclic compound will contain, inclusive of all substituents, no more than 50 about carbon atoms, more usually no more than about 30 to 40 carbon atoms.

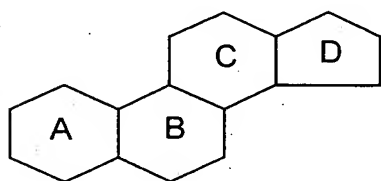
The polycyclic compound can comprise at least two ring components, or least three ring components, or least four ring components.

Preferably, the polycyclic compound comprises four ring components.

5

Preferred polycyclic compounds have a steroidal ring component - that is to say a cyclopentanophenanthrene skeleton, or bio-isosteres thereof.

As is well known in the art, a classical steroidal ring structure has the generic formula of:

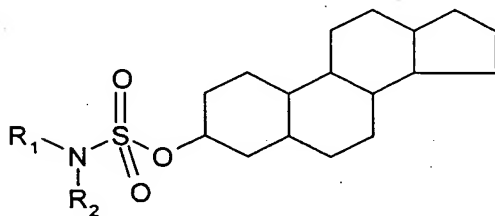


10

In the above formula, the rings have been labelled in the conventional manner.

When the sulphamate compound has a steroidal ring component the compound may be a compound having the formula

15



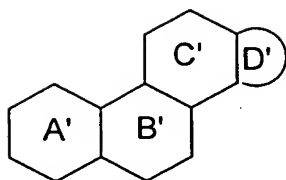
wherein each of  $R_1$  and  $R_2$  is independently selected from H or a hydrocarbyl group.

An example of a bio-isostere is when any one or more of rings A, B, C and D is a heterocyclic ring and/or when any one or more of rings A, B, C and D has been substituted and/or when any one or more of rings A, B, C and D has been modified; but wherein the bio-isostere in the absence of the sulphamate group has steroidal properties.

20

In this regard, the structure of a preferred polycyclic compound can be presented as:





wherein each ring A', B', C' and D' independently represents a heterocyclic ring or a non-heterocyclic ring, which rings may be independently substituted or unsubstituted, saturated or unsaturated.

5

By way of example, any one or more of rings A', B', C' and D' may be independently substituted with suitable groups - such as an alkyl group, an allyl group, an hydroxy group, a halo group, a hydrocarbyl group, an oxyhydrocarbyl group etc.

- 10 An example of D' is a five or six membered non-heterocyclic ring having at least one substituent.

In one preferred embodiment, the ring D' is substituted with a ethinyl group.

- 15 If any one of rings A', B', C' and D' is a heterocyclic ring, then preferably that heterocyclic ring comprises a combination of C atoms and at least one N atom and/or at least one O atom. Other heterocyclic atoms may be present in the ring.

- 20 Examples of suitable, preferred steroidal nuclei rings A'-D' of the compounds of the present invention include rings A-D of oestrone and dehydroepiandrosterone.

Preferred steroidal nuclei rings A'-D' of the compounds of the present invention include rings A-D of:

- 25 oestrones and substituted oestrones, viz:

oestrone

2-OH-oestrone

2-alkoxy-oestrone (such as C<sub>1-6</sub> alkoxy-oestrone, such as 2-methoxy-oestrone)

2-alkyl-oestrone (such as C<sub>1-6</sub> alkyl-oestrone, such as 2-ethyl-oestrone)

- 30 4-OH-oestrone

6 $\alpha$ -OH-oestrone

7 $\alpha$ -OH-oestrone

16 $\alpha$ -OH-oestrone

16 $\beta$ -OH-oestrone

oestradiols and substituted oestradiols, viz:

5 2-OH-17 $\beta$ -oestradiol

2-alkoxy-17 $\beta$ -oestradiol (such as C<sub>1-6</sub> alkoxy-17 $\beta$ -oestradiol, such as 2-methoxy-17 $\beta$ -oestradiol)

2-alkyl-17 $\beta$ -oestradiol (such as C<sub>1-6</sub> alkyl-17 $\beta$ -oestradiol, such as 2-ethyl-17 $\beta$ -oestradiol)

4-OH-17 $\beta$ -oestradiol

10 6 $\alpha$ -OH-17 $\beta$ -oestradiol

7 $\alpha$ -OH-17 $\beta$ -oestradiol

2-OH-17 $\alpha$ -oestradiol

2-alkoxy-17 $\alpha$ -oestradiol (such as C<sub>1-6</sub> alkoxy-17 $\alpha$ -oestradiol, such as 2-methoxy-17 $\alpha$ -oestradiol)

15 2-alkyl-17 $\alpha$ -oestradiol (such as C<sub>1-6</sub> alkyl-17 $\alpha$ -oestradiol, such as 2-ethyl-17 $\alpha$ -oestradiol)

4-OH-17 $\alpha$ -oestradiol

6 $\alpha$ -OH-17 $\alpha$ -oestradiol

7 $\alpha$ -OH-17 $\alpha$ -oestradiol

16 $\alpha$ -OH-17 $\alpha$ -oestradiol

20 16 $\alpha$ -OH-17 $\beta$ -oestradiol

16 $\beta$ -OH-17 $\alpha$ -oestradiol

16 $\beta$ -OH-17 $\beta$ -oestradiol

17 $\alpha$ -oestradiol

17 $\beta$ -oestradiol

25 17 $\alpha$ -ethinyl-17 $\beta$ -oestradiol

17 $\beta$ -ethinyl-17 $\alpha$ -oestradiol

oestriols and substituted oestriols, viz:

oestriol

30 2-OH-oestriol

2-alkoxy-oestriol (such as C<sub>1-6</sub> alkoxy-oestriol, such as 2-methoxy-oestriol)

2-alkyl-oestriol (such as C<sub>1-6</sub> alkyl-oestriol, such as 2-ethyl-oestriol)

4-OH-oestriol

6 $\alpha$ -OH-oestriol

7 $\alpha$ -OH-oestriol

dehydroepiandrosterones and substituted dehydroepiandrosterones, viz:

dehydroepiandrosterones

- 5 6 $\alpha$ -OH-dehydroepiandrosterone
  - 7 $\alpha$ -OH-dehydroepiandrosterone
  - 16 $\alpha$ -OH-dehydroepiandrosterone
  - 16 $\beta$ -OH-dehydroepiandrosterone
- 10 In general terms the ring system A'B'C'D' may contain a variety of non-interfering substituents. In particular, the ring system A'B'C'D' may contain one or more hydroxy, alkyl especially lower (C<sub>1</sub>-C<sub>6</sub>) alkyl, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl and other pentyl isomers, and n-hexyl and other hexyl isomers, alkoxy especially lower (C<sub>1</sub>-C<sub>6</sub>) alkoxy, e.g. methoxy, ethoxy, propoxy etc., alkynyl, e.g. ethynyl, or
- 15 halogen, e.g. fluoro substituents.

In an alternative embodiment, the polycyclic compound may not contain or be based on a steroid nucleus. In this regard, the polycyclic compound may contain or be based on a non-steroidal ring system - such as diethylstilboestrol, stilboestrol, coumarins, and other

20 ring systems. Other suitable non-steroidal compounds for use in or as the composition of the present invention may be found in US-A-5567831.

In a preferred aspect the at least one sulphamate group is attached to any one or more of the ring components of a cyclic compound.

25 Preferably, the polycyclic compound has a steroidal structure and wherein the sulphamate group is attached to the A ring.

Preferably, the sulphamate group is attached to the 3 position of the A ring.

30 Preferably the sulphamate compound comprises at least one hydrocarbyl group or oxyhydrocarbyl group. The hydrocarbyl group or oxyhydrocarbyl group are referred to herein as a (oxy)hydrocarbyl group.

35 In a preferred embodiment the (oxy)hydrocarbyl group and the sulphamate group are

each attached to the same ring at positions *ortho* with respect to each other.

A preferred sulphamate compound is an (oxy)hydrocarbyl steroidal sulphamate compound (i.e. a sulphamate compound comprising a steroidal component and an  
5 (oxy)hydrocarbyl group). In this aspect preferably the (oxy)hydrocarbyl group and the sulphamate group are each attached to the A ring of the steroidal structure/component.

In respect of steroidal embodiments of the invention preferably the (oxy)hydrocarbyl group is attached to the 2 position of the A ring of the steroidal structure and/or the  
10 sulphamate group is attached to the 3 position of the A ring of the steroidal structure. In one embodiment, preferably, the sulphamate compound is an (oxy)hydrocarbyl steroidal sulphamate compound wherein the sulphamate group is in the 3 position on the steroidal component and/or the (oxy)hydrocarbyl group is in the 2-position position on the steroidal component.

15

In one embodiment, preferably, the sulphamate compound is an oxyhydrocarbyl derivative of oestrone sulphamate.

In one embodiment, preferably, the sulphamate compound is an oxyhydrocarbyl  
20 derivative of oestrone-3-O-sulphamate.

In one embodiment, preferably, the sulphamate compound is a C<sub>1-6</sub> (such as a C<sub>1-3</sub>) alkoxy or alkyl derivative of oestrone-3-O-sulphamate.

25 In one embodiment, preferably, the sulphamate compound is a 2-C<sub>1-6</sub> (such as a C<sub>1-3</sub>) alkoxy or alkyl derivative of oestrone-3-O-sulphamate.

The term "oxyhydrocarbyl group" as used herein means a group comprising at least C, H and O and may optionally comprise one or more other suitable substituents. Examples of  
30 such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the oxyhydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the  
35 oxyhydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent

to those skilled in the art and include, for instance, sulphur and nitrogen.

In one preferred embodiment of the present invention, the oxyhydrocarbyl group is a oxyhydrocarbon group.

5

Here the term "oxyhydrocarbon" means any one of an alkoxy group, an oxyalkenyl group, an oxyalkynyl group, which groups may be linear, branched or cyclic, or an oxyaryl group. The term oxyhydrocarbon also includes those groups but wherein they have been optionally substituted. If the oxyhydrocarbon is a branched structure having  
10 substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

15

Preferably, the (oxy)hydrocarbyl group is an alkoxy/alkyl. The alkyl group (of the alkoxy substituent) is preferably a lower alkyl group containing from 1 to 5 carbon atoms, that is to say methyl, ethyl, propyl etc. Preferably, the alkyl group is methyl or ethyl.

20

In one embodiment, preferably the oxyhydrocarbyl group is a group of the formula  $C_{1-6}O$  (such as a  $C_{1-3}O$ ), more preferably a methoxy group.

More preferably the group  $C_{1-6}O$  is attached to the 2 position of the A ring of a steroidal nucleus.

25

In a highly preferred embodiment the sulphamate compound is 2-methoxyoestrone-3-O-sulphamate.

In one embodiment, preferably the hydrocarbyl group is a group of the formula  $C_{1-6}$ , more preferably an ethyl group.

30

In a highly preferred embodiment the sulphamate compound is 2-ethyloestrone-3-O-sulphamate.

If the sulphamate compound comprises a steroidal nucleus, preferably the A ring has an (oxy)hydrocarbyl group at the 2 position.

35

Preferably the sulphamate compound comprises at least one sulfanyhydrocarbyl group. By the term sulfanyhydrocarbyl it is meant a group of the formula  $-L-S-R$ , wherein L is an optional linker group, S represents sulphur and R is a hydrocarbyl group (as defined herein).

5

In a preferred embodiment the sulfanyhydrocarbyl group and the sulphamate group are each attached to the same ring at positions *ortho* with respect to each other.

10

A preferred sulphamate compound is an sulfanyhydrocarbyl steroidal sulphamate compound (i.e. a sulphamate compound comprising a steroidal component and an sulfanyhydrocarbyl group). In this aspect preferably the sulfanyhydrocarbyl group and the sulphamate group are each attached to the A ring of the steroidal structure/component.

15

In respect of steroidal embodiments of the invention preferably the sulfanyhydrocarbyl group is attached to the 2 position of the A ring of the steroidal structure and/or the sulphamate group is attached to the 3 position of the A ring of the steroidal structure. In one embodiment, preferably, the sulphamate compound is an sulfanyhydrocarbyl steroidal sulphamate compound wherein the sulphamate group is in the 3 position on the steroidal component and/or the sulfanyhydrocarbyl group is in the 2-position position on the steroidal component.

20

In one embodiment, preferably, the sulphamate compound is an sulfanyhydrocarbyl derivative of oestrone sulphamate.

25

In one embodiment, preferably, the sulphamate compound is an sulfanyhydrocarbyl derivative of oestrone-3-O-sulphamate.

30

In one embodiment, preferably, the sulphamate compound is a  $C_{1-6}$  (such as a  $C_{1-3}$ ) alkyl sulfanyl derivative of oestrone-3-O-sulphamate.

In one embodiment, preferably, the sulphamate compound is a 2- $C_{1-6}$  (such as a  $C_{1-3}$ ) alkyl sulfanyl derivative of oestrone-3-O-sulphamate.

35

In one preferred embodiment of the present invention, the sulfanyhydrocarbyl group is a sulfanyhydrocarbon group.

By the term "sulfanyhydrocarbon" it is meant a group of the formula  $-L-S-R$ , wherein L is an optional linker group, S represents sulphur and R is a hydrocarbon group (as defined herein).

5

In one embodiment, preferably the sulfanyhydrocarbonyl group is a group of the formula  $C_{1-6}S$  (such as a  $C_{1-3}S$ ), more preferably a  $CH_3S$  or  $CH_3CH_2S$  group.

10

More preferably the group  $C_{1-6}S$  is attached to the 2 position of the A ring of a steroidal nucleus.

In a highly preferred embodiment the sulphamate compound is 2-methylsulfanyloestrone-3-O-sulphamate or 2-ethylsulfanyloestrone-3-O-sulphamate.

15

Preferably, if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound then the sulphate compound would be hydrolysable by a steroid sulphatase enzyme (E.C.3.1.6.2).

20

Preferably if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound and the sulphate compound were to be incubated with a steroid sulphatase enzyme (E.C.3.1.6.2) at a pH 7.4 and  $37^\circ C$  it would provide a  $K_m$  value of less than 50 mM.

25

Preferably if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound and the sulphate compound were to be incubated with a steroid sulphatase enzyme (E.C.3.1.6.2) at a pH 7.4 and  $37^\circ C$  it would provide a  $K_m$  value of less than 50  $\mu M$ .

30

Preferably the sulphamate compound is suitable for use as an inhibitor of oestrone sulphatase (E.C. 3.1.6.2).

In one preferred embodiment of the present invention, preferably the sulphamate compound is non-oestrogenic. The term "non-oestrogenic" means exhibiting no or substantially no oestrogenic activity.

35

In one preferred embodiment of the present invention, preferably the sulphamate compound are not capable of being metabolised to compounds which display or induce hormonal activity.

- 5 In one preferred embodiment of the present invention, preferably the composition of the present invention is orally active.

The present invention is based on the highly surprising finding that the combination of a sulphamate compound and a apoptosis inducer provides an effective treatment of  
10 cancer.

More in particular, we have surprisingly found that the composition of the present invention can induce apoptosis.

- 15 We have identified that in some aspects it may not be necessary for the compound to be a sulphamate compound. Thus in a further aspect the present invention provides a composition comprising (i) a steroidal compound; and (ii) an apoptosis inducer. In this aspect the preferred features of the an apoptosis inducer and steroid nucleus of sulphamate compound described above equally apply to the composition comprising the  
20 steroidal compound and apoptosis inducer. The composition comprising (i) a steroidal compound; and (ii) an apoptosis inducer may be utilised in each of the uses and methods described herein.

Apoptosis is induced by MT-targeting drugs, a process which may involve the  
25 phosphorylation (and inactivation) of the apoptosis regulator, the bcl-2 protein (Halder, Cancer Res. 57: 229, 1997).

Preferably the composition of the present invention further comprises a pharmaceutically acceptable carrier, diluent, or excipient.

30

For pharmaceutical administration, the composition of the present invention can be formulated in any suitable manner utilising conventional pharmaceutical formulating techniques and pharmaceutical carriers, adjuvants, excipients, diluents etc. - such as those for parenteral administration. Approximate effective dose rates are in the range 100 to 800  
35 mg/day depending on the individual activities of the compounds in question and for a



patient of average (70Kg) bodyweight. More usual dosage rates for the preferred and more active compositions will be in the range 200 to 800 mg/day, more preferably, 200 to 500 mg/day, most preferably from 200 to 250 mg/day. They may be given in single dose regimes, split dose regimes and/or in multiple dose regimes lasting over several days. For oral administration they may be formulated in tablets, capsules, solution or suspension containing from 100 to 500 mg of composition per unit dose. Alternatively and preferably the compositions will be formulated for parenteral administration in a suitable parenterally administrable carrier and providing single daily dosage rates in the range 10 to 800 mg, 10 to 500 mg, 200 to 800 mg, preferably 200 to 500, more preferably 200 to 250 mg. Such effective daily doses will, however, vary depending on inherent activity of the active ingredient and on the bodyweight of the patient, such variations being within the skill and judgement of the physician.

The composition or compound of the present invention may be administered in any suitable manner - such as any one or more of oral administration, topical administration (such as by means of a patch), parenteral administration, rectal administration or by inhalation spray.

In the method of treatment, the subject is preferably a mammal, more preferably a human. For some applications, preferably the human is a woman.

For particular applications, it is envisaged that the compositions of the present invention may be used in combination therapies, either with another sulphatase inhibitor, or, for example, in combination with an aromatase inhibitor, such as for example, 4-hydroxyandrostenedione (4-OHA).

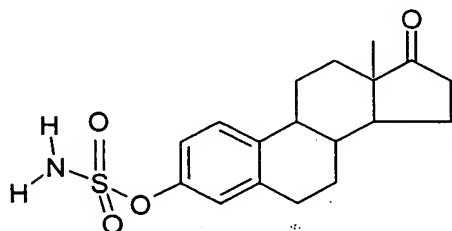
In accordance with the present invention, the components of the composition can be added in admixture, simultaneously or sequentially. Furthermore, in accordance with the present invention it may be possible to form at least a part of the composition *in situ* (such as *in vivo*) by inducing the expression of - or increasing the expression of - one of the components. For example, it may be possible to induce the expression of - or increase the expression of - the apoptosis inducer, such as TNF. By way of example, it may be possible to induce the expression of - or increase the expression of - TNF by adding bacterial lipopolysaccharide (LPS) and muramyl dipeptide (MDP). In this regard, bacterial LPS and MDP in combination can stimulate TNF production from murine spleen

cells *in vitro* and tumour regression *in vivo* (Fuks *et al* Biull Eksp Biol Med 1987 104: 497-499).

Examples of suitable sulphamate compounds for use in or as the composition of the present invention, or examples of suitable compounds that can be converted to suitable sulphamate compounds for use in or as the composition of the present invention, can be found in the art - such as PCT/GB92/01587, PCT/GB97/03352, PCT/GB97/00444, GB 9725749.7, GB 9725750.5, US-A-5567831, US-A-5677292, US-A-5567831, WO-A-96/05216, and WO-A-96/05217.

By way of example, PCT/GB92/01587 teaches novel steroid sulphatase inhibitors and pharmaceutical compositions containing them for use in the treatment of oestrone dependent tumours, especially breast cancer. These steroid sulphatase inhibitors are sulphamate esters. Examples of such inhibitors are sulphamate ester derivatives of steroids.

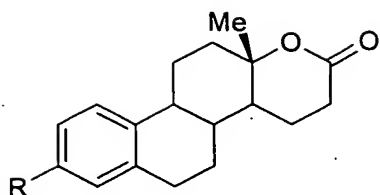
A compound suitable for use in the present invention - which is also a preferred compound of PCT/GB92/01587 - is oestrone-3-sulphamate (otherwise known as "EMATE"), which has the following structure:



It is known that EMATE is a potent E1-STS inhibitor as it displays more than 99% inhibition of E1-STS activity in intact MCF-7 cells at 0.1  $\mu$ M. EMATE also inhibits the E1-STS enzyme in a time- and concentration-dependent manner, indicating that it acts as an active site-directed inactivator.

Preferably, the A ring has a substituent that is an (oxy)hydrocarbyl group.

Another compound suitable for use in the present invention has at least the following skeletal structure:

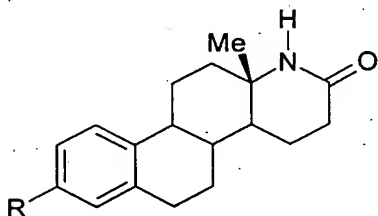


wherein R denotes a sulphonamide group as described above:

Preferably, R is the above-mentioned preferred formula for the sulphonamide group. In this regard, it is preferred that at least one of  $R_1$  and  $R_2$  is H.

Preferably, the A ring has a substituent that is an (oxy)hydrocarbyl group.

Another compound suitable for use in the present invention has at least the following skeletal structure:



wherein R denotes a sulphonamide group as described above.

Preferably, R is the above-mentioned preferred formula for the sulphonamide group. In this regard, it is preferred that at least one of  $R_1$  and  $R_2$  is H.

Preferably, the A ring has a substituent that is an (oxy)hydrocarbyl group.

In accordance with a preferred aspect of the present invention, if the sulphonamide group of the compound were to be replaced with a sulphate group to form a sulphate compound then that sulphate compound would be hydrolysable by an enzyme having steroid sulphonatase (E.C. 3.1.6.2) activity - i.e. when incubated with steroid sulphonatase EC 3.1.6.2 at pH 7.4 and 37°C.

In one preferred embodiment, if the sulphonamide group of the compound were to be replaced with a sulphate group to form a sulphate compound then that sulphate compound would be hydrolysable by an enzyme having steroid sulphonatase (E.C. 3.1.6.2) activity and would yield a  $K_m$  value of less than 50mM when incubated with steroid sulphonatase EC

3.1.6.2 at pH 7.4 and 37°C.

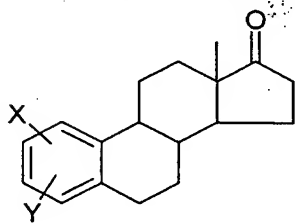
In another preferred embodiment, if the sulphamate group of the compound were to be replaced with a sulphate group to form a sulphate compound then that sulphate compound would be hydrolysable by an enzyme having steroid sulphatase (E.C. 3.1.6.2) activity and would yield a  $K_m$  value of less than 50 $\mu$ M when incubated with steroid sulphatase EC 3.1.6.2 at pH 7.4 and 37°C.

In a further aspect the present invention provides use of a sulphamate compound for the manufacture of a medicament to prevent and/or inhibit tumour growth; wherein the sulphamate compound is suitable for use as an inhibitor of oestrone sulphatase (E.C. 3.1.6.2); wherein the compound is a polycyclic compound having a steroidal structure, or a bio-isostere thereof; wherein the polycyclic compound comprises at least one sulphamate group attached to the A ring; and wherein the polycyclic compound comprises at least one oxyhydrocarbyl group attached to the A ring.

We have found that sulphamate compounds upregulate a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor.

Thus in a further aspect the present invention provides use of a sulphamate compound in the manufacture of a medicament to upregulate a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor.

A preferred sulphamate compound of the present invention has the formula:



wherein X is an (oxy)hydrocarbyl group; and Y is a sulphamate group; and wherein rings A, B, C and D are independently optionally substituted.

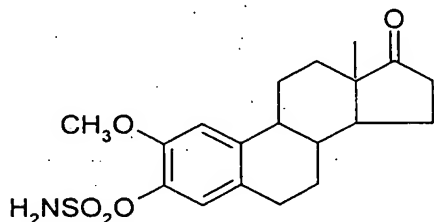
Preferably Y is in the 3-position.

Preferably X is in the 2-position.

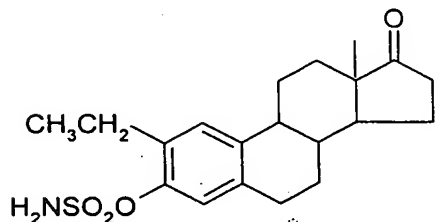
For the present invention, preferably the sulphamate compound is an oxyhydrocarbonyl steroidal sulphamate compound, in particular 2-methoxyoestrone-3-O-sulphamate or 2-ethyloestrone-3-O-sulphamate, or a pharmaceutically active salt thereof, including analogues thereof.

2-methoxyoestrone-3-O-sulphamate is an analogue of EMATE - and can be called 2-methoxy EMATE (2-MeOEMATE). 2-ethyloestrone-3-O-sulphamate is an analogue of EMATE - and can be called 2-ethyl EMATE (2-EtEMATE). 2-MeOEMATE and 2-EtEMATE are described in detail in WO 99/64013 and WO 00/66095, respectively.

2-methoxy EMATE has the formula presented as formula below:



2-ethyl EMATE has the formula presented as formula below:



The sulphamate compounds of the present invention may be prepared by reacting an appropriate alcohol with the appropriate sulfamoyl chloride,  $R_1R_2NSO_2Cl$ . Preferred conditions for carrying out the reaction are as follows. Sodium hydride and a sulfamoyl chloride are added to a stirred solution of the alcohol in anhydrous dimethyl formamide at  $0^\circ C$ . Subsequently, the reaction is allowed to warm to room temperature whereupon stirring is continued for a further 24 hours. The reaction mixture is poured onto a cold saturated solution of sodium bicarbonate and the resulting aqueous phase is extracted with dichloromethane. The combined organic extracts are dried over anhydrous  $MgSO_4$ . Filtration followed by solvent evaporation *in vacuo* and co-evaporated with toluene affords a crude residue which is further purified by flash chromatography. Preferably, the alcohol is

derivatised, as appropriate, prior to reaction with the sulfamoyl chloride. Where necessary, functional groups in the alcohol may be protected in known manner and the protecting group or groups removed at the end of the reaction.

- 5 In summation, the present invention provides compositions for use in treatment of tumours and pharmaceutical compositions containing them.

The present invention will now be described only by way of example, in which reference, in which reference shall be made to the following Figures.

10

Figure 1A shows a graph of the effect of 2-MeOEMATE on TRAIL induced apoptosis in CAL51 cells;

Figure 1B shows a graph of the effect of 2-EtEMATE on TRAIL induced apoptosis in CAL51 cells;

- 15 Figure 1C shows a graph of the effect of 2-MeOEMATE on TRAIL induced apoptosis in MCF-7 cells;

Figure 1D shows a graph of the effect of 2-EtEMATE on TRAIL induced apoptosis in MCF-7 cells ; and

- 20 Figure 2 shows a graph of the effect of 2-MeOEMATE and 2-EtEMATE on DR4 and DR5 mRNA levels in Cal51 cells.

- Figures 1A to 1D show effects of 2-MeOEMATE and 2-EtEMATE on TRAIL induced apoptosis in CAL51 and MCF-7 cells. Cell proliferation assays in which CAL51 cells or MCF7 cells were plated at a density of 5,000 in 96 well plates and treated with 0, 0.05, 0.5 or 5uM MeoEMATE or 2-EtEMATE in the presence of 0, 50 or 250ng/ml recombinant human TRAIL. Results are the mean of triplicates +/- S.D. and are expressed as a percentage of results for untreated cells.

- 30 Figure 2 shows the effect of 2-MeOEMATE and 2-EtEMATE on DR4 and DR5 mRNA levels in Cal51 cells. RNase protection assay showing DR5, DR4 and GAPDH mRNA levels at 0, 8, 16, 24 and 48 hours following treatment with 0.5uM 2-MeOEMATE and 2-EtEMATE in Cal51 cells. Graph shows quantification of data from phosphoimager analysis of DR5 and DR4 bands normalised by expression as ratio of DR5/GAPDH.

## **EXAMPLES**

2-MeOEMATE and 2-EtEMATE were synthesised in accordance with the teachings of WO 99/64013 and WO 00/66095, respectively.

### **Example 1**

We investigated if 2-MeOEMATE and 2-EtEMATE would co-operate with TRAIL to induce apoptosis in breast cancer cells. The data obtained are shown in Figures 1A to 1D.

#### **Procedure**

Cell proliferation was measured using the Cell Titre 96 Aqueous One Solution Cell proliferation assay (Promega). This is a colorimetric method which determines cell viability and can therefore be used to measure the ability of drugs to cause cell death. The assay is performed by adding the cell proliferation reagent directly to the cells. Metabolically active cells convert the reagent to a coloured formazan product and the absorbance at 490nm is measured 1-4hrs after incubation at 37°C.

For the TRAIL co-operation experiments CAL-51 or MCF7 cells were plated at 5,000 cells per well in 96 well plates and treated with 0, 0.05, 0.5 or 5uM 2-MeOEMATE or 2-EtEMATE in the presence of 0, 50 or 250ng/ml recombinant human TRAIL. Cell proliferation was measured 2 days (CAL-51) or 4 days (MCF-7) after addition of drugs. Tests were performed in triplicate and data are means of triplicate results +/-S.D. and are expressed as a percentage of results obtained for untreated cells. Experiments on CAL51 cells were performed 4 times for each compound and once for each compound on MCF7s.

#### **Results**

Treatment with 2-MeOEMATE or 2-EtEMATE alone induced up to 60% cell death in CAL51cells and MCF-7 cells over 2 and 4 days respectively. When treated with TRAIL alone CAL51 cells were resistant to TRAIL induced cell death. However treatment with 2-MeOEMATE or 2-EtEMATE in combination with TRAIL in CAL51 cells enhanced the amount of cell death compared to treatment with 2-MeOEMATE or 2-EtEMATE alone.

Therefore the sulfamoylated derivatives sensitised CAL51 cells to TRAIL induced apoptosis in a co-operative manner.

In contrast MCF-7 cells were sensitive to TRAIL induced apoptosis when treated with TRAIL alone and co-treatment with 2-MeOEMATE or 2-EtEMATE did not enhance cell death further compared to treatment of cells with any of the drugs alone.

2-MeOEMATE and 2-EtEMATE enhance TRAIL induced apoptosis in Cal51 cells but not MCF-7 cells.

### Example 2

We investigated if the co-operation observed between TRAIL and 2-MeOEMATE or 2-EtEMATE to induce cell death in CAL51 cells was due to upregulation of DR4 and/or DR5 receptors by 2-MeOEMATE and 2-EtEMATE. We used RNase protection assays to examine the levels of mRNAs for DR4 and DR5 following treatment of CAL51 cells with 2-MeOEMATE and 2-EtEMATE over 48 hours. The data obtained are shown in Figure 2.

### **Procedure**

This experiment was performed once. A radiolabeled RNA multi-probe was synthesised (Pharmingen RiboQuant Human Apoptosis Multiprobe template set hAPO-3c) using [ $\alpha^{32}\text{P}$ ] UTP and T7 RNA polymerase. This multi-probe template set allows simultaneous generation of several probes for detection and measurement of apoptosis related genes. The set also includes probes for the housekeeping genes, L32 and GAPDH, which allows for normalization of sampling and technique errors to permit comparison of mRNA levels between samples.

Total RNA was prepared using Trizol reagent (Gibco-BRL) from CAL-51 cells treated with 2-MeOEMATE or 2-EtEMATE for 0, 8, 16, 24 and 48hrs. The radiolabelled multi-probe was hybridised to the RNAs overnight. The unhybridised RNA was digested with RNase and the hybridised (protected) fragments were separated on a 6% acrylamide gel. The gel was dried and analysed by phosphoimaging on the Personal Molecular Imager Fx (Biorad). Protected fragment bands corresponding to DR5 and GAPDH were quantified and expressed as a ratio of DR5/GAPDH to normalize results.



## Results

DR5 mRNA levels were significantly increased between 24 and 48 hours following treatment with both drugs compared to the untreated control. In contrast DR4 mRNA levels were low in comparison to DR5 levels and did not change significantly over this time period.

2-MeOEMATE and 2-EtEMATE upregulate DR5 receptor mRNA levels in CAL-51 cells.

## SUMMARY

We have demonstrated that when used in combination with apoptosis inducers such as TRAIL, sulphamate compounds such as 2-MeOEMATE and 2-EtEMATE increase the sensitivity of breast cancer cells to apoptosis inducers. The mechanism for this increased sensitivity may involve the upregulation of receptor levels, for example DR5 mRNA receptor levels, induced by the sulfamoylated derivatives.

Thus, in summary, the present invention provides a composition and compound suitable for use in the treatment of cancers and, especially, breast cancer.

In particular, in one aspect the present invention addresses the problem of blocking the growth of tumours in endocrine-dependent tissues (e.g. breast, endometrium, prostate). Nevertheless, other tumours (e.g. sarcomas, melanomas) should also be amenable to treatment with the composition and compound of the present invention.

It is also believed that the present invention has implications in treating hormonal conditions in addition to those associated with oestrogen. Hence, the present invention also provides a composition that is capable of affecting hormonal activity and is capable of affecting an immune response, wherein the composition is the composition of the present invention.

It is also to be understood that the composition of the present invention may have other important medical implications.

For example, the composition of the present invention may be useful in the treatment of the disorders listed in WO-A-98/05635. For ease of reference, part of that list is now provided: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

In addition, or in the alternative, the composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/07859. For ease of reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration; inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating haemophilia and stroke); antiinflammatory activity (for treating e.g. septic shock or Crohn's disease); as antimicrobials; modulators of e.g. metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine.

In addition, or in the alternative, the composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/09985. For ease of reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-

inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells;

5 inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular

10 inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal

15 diseases, periodontal diseases or other dental diseases, orchitis or epididymo-orchitis, infertility, orchidial trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic

20 neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fundus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune

25 and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's

30 disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy,

35 chronic neuropathy, Guillain-Barre syndrome, Sydenham chora, myasthenia gravis,

pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

15

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

25

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5 Chinnaiyan AM et al., 2000 Proc Natl Acad Sci USA 97: 1754-1759

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Huang P et al., 2000 Nature 407 390-395

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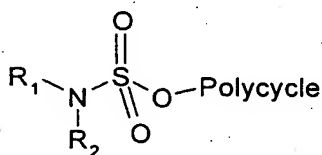
Keane MM et al., 1999 Cancer Res 59: 734-41

MacCarthy-Morrogh et al., 2000 Cancer Res 60: 5441-50

15 Wu GS et al., 1997 Nat Genet 17: 141-143

**CLAIMS**

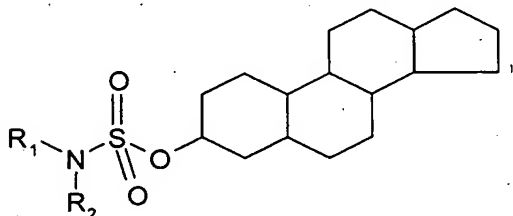
1. A composition comprising
  - i) a compound comprising a sulphamate group ("a sulphamate compound"); and
  - 5 ii) an apoptosis inducer.
2. A composition according to claim 1 wherein the apoptosis inducer is an apoptosis inducing ligand.
- 10 3. A composition according to claim 1 wherein the apoptosis inducer is an apoptosis inducing cytokine.
4. A composition according to claim 1 wherein the apoptosis inducer is a tumour necrosis factor apoptosis inducing ligand (TRAIL).
- 15 5. A composition according to claim 4 wherein the TRAIL is TRAIL/Apo-2L.
6. A composition according to any one of the preceding claims wherein the apoptosis inducer is capable of interacting with a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor.
- 20 7. A composition according to claim 6 wherein the receptor is DR4 and/or DR5.
8. A composition according to any one of the preceding claims wherein the sulphamate compound is a cyclic compound.
- 25 9. A composition according to any one of the preceding claims wherein the sulphamate compound is a polycyclic compound.
- 30 10. A composition according to claim 9 wherein the sulphamate compound is a compound having the formula



wherein each of R<sub>1</sub> and R<sub>2</sub> is independently selected from H or a hydrocarbonyl group.

11. A composition according to any one of the preceding claims wherein the sulphamate compound has a steroidal structure.

12. A composition according to claim 11 wherein the sulphamate compound is a compound having the formula



wherein each of  $R_1$  and  $R_2$  is independently selected from H or a hydrocarbyl group.

13. A composition according to claim 11 or 12 wherein the sulphamate compound has at least one sulphamate group attached to the 3 position of the A ring of the steroidal nucleus.

14. A composition according to any one of the preceding claims wherein the sulphamate compound is substituted with a hydrocarbyl or an oxyhydrocarbyl group.

15. A composition according to claim 14 wherein the (oxy)hydrocarbyl group and the sulphamate group are each attached to the same ring at positions *ortho* with respect to each other.

16. A composition according to claim 15 wherein the sulphamate compound has a steroidal structure and the (oxy)hydrocarbyl group and the sulphamate group are each attached to the A ring of the steroidal structure.

17. A composition according to claim 16 wherein the (oxy)hydrocarbyl group is attached to the 2 position of the A ring of the steroidal structure.

18. A composition according to claim 16 or 17 wherein the sulphamate group is attached to the 3 position of the A ring of the steroidal structure.

19. A composition according to any one of claims 14 to 18 wherein the

oxyhydrocarbyl group is a group of the formula  $C_{1-6}O$ .

20. A composition according to claim 19 wherein the group  $C_{1-6}O$  is a methoxy group.

5 21. A composition according to claim 1 wherein the sulphamate compound is 2-methoxyoestrone-3-O-sulphamate.

22. A composition according to any one of claims 14 to 18 wherein the hydrocarbyl group is a group of the formula  $C_{1-6}$ .

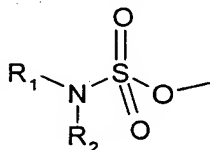
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23. A composition according to claim 22 wherein the group  $C_{1-6}$  is an ethyl group

24. A composition according to claim 1 wherein the sulphamate compound is 2-ethyloestrone-3-O-sulphamate.

15

25. A composition according to any one of the preceding claims wherein the sulphamate group of the sulphamate compound has the formula:



wherein each of  $R_1$  and  $R_2$  is independently selected from H or a hydrocarbyl group.

20

26. A composition according to any one of the preceding claims wherein the sulphamate compound is suitable for use as an inhibitor of oestrone sulphatase (E.C. 3.1.6.2).

25 27. A composition according to any one of the preceding claims wherein if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound then the sulphate compound would be hydrolysable by a steroid sulphatase enzyme (E.C.3.1.6.2).

30 28. A composition according to any one of the preceding claims wherein if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound and incubated with a steroid sulphatase enzyme (E.C.3.1.6.2) at a pH 7.4 and 37°C it would provide a  $K_m$  value of less than 50 mM.



29. A composition according to any one of the preceding claims wherein if the sulphamate group on the sulphamate compound were to be replaced with a sulphate group to form a sulphate compound and incubated with a steroid sulphotase enzyme (E.C.3.1.6.2) at a pH 7.4 and 37°C it would provide a  $K_m$  value of less than 50  $\mu M$ .
30. A composition according to any one of the preceding claims, wherein the composition further comprises a pharmaceutically acceptable carrier, diluent, or excipient.
31. A composition according to any one of the preceding claims for use in medicine.
32. Use of a composition according to any one of the preceding claims in the manufacture of a medicament to prevent and/or inhibit tumour growth.
33. Use of a composition according to any one of the preceding claims in the manufacture of a medicament to induce apoptosis.
34. Use of an sulphamate compound in the manufacture of a medicament to upregulate a tumour necrosis factor apoptosis inducing ligand (TRAIL) receptor.
35. Use according to claim 34 wherein the receptor is DR4 and/or DR5.
36. Use according to claim 34 or 35 wherein the sulphamate compound is a compound defined in any one of claims 1 to 29.
37. A method of treatment comprising administering to a subject in need of treatment a composition according to any one of the claims 1 to 30.
38. A method of treatment comprising administering to a subject in need of treatment a composition according to any one of claims 1 to 30 or an sulphamate compound in order to induce apoptosis.
39. A composition substantially as described herein.

**ABSTRACT****COMPOSITION**

- 5 The present invention provides a composition comprising (i) a compound comprising a sulphamate group ("a sulphamate compound"); and (ii) an apoptosis inducer.

Figure 1A

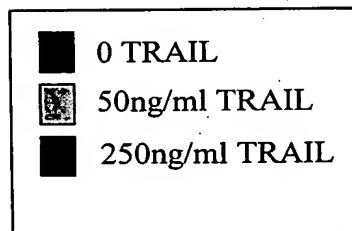
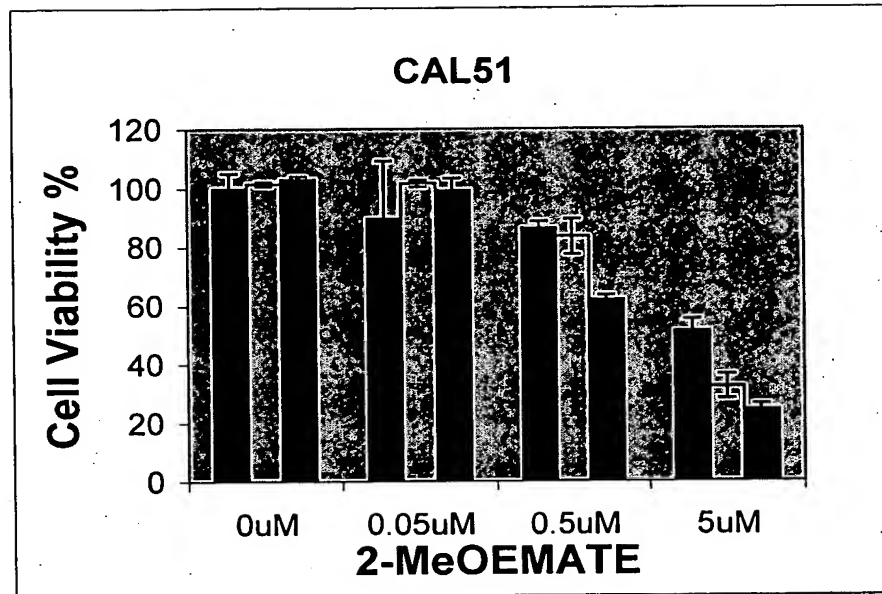


Figure 1A



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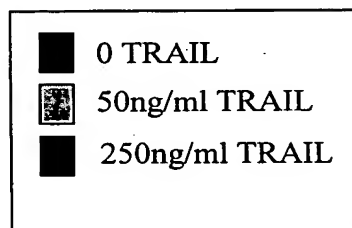
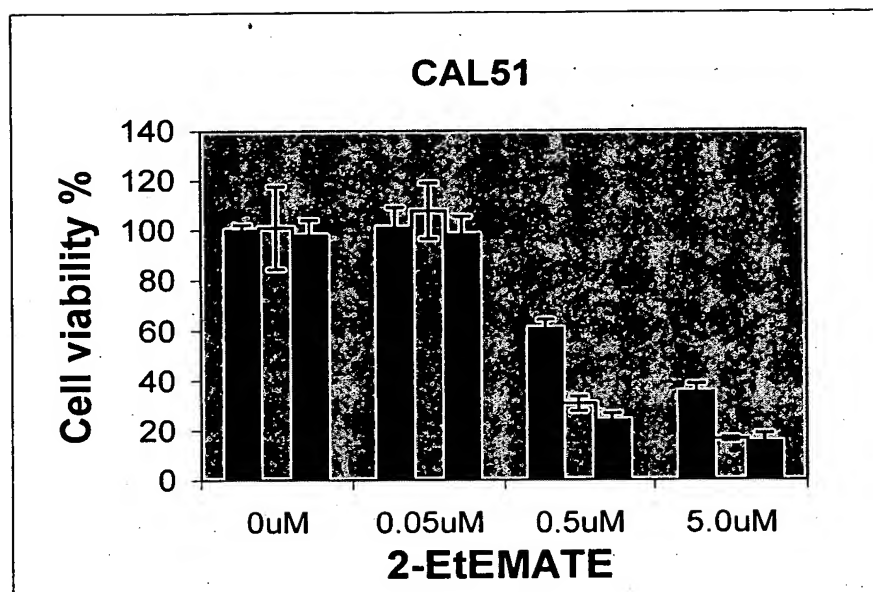


Figure 1B



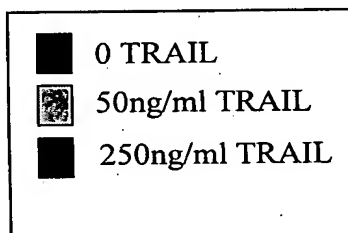
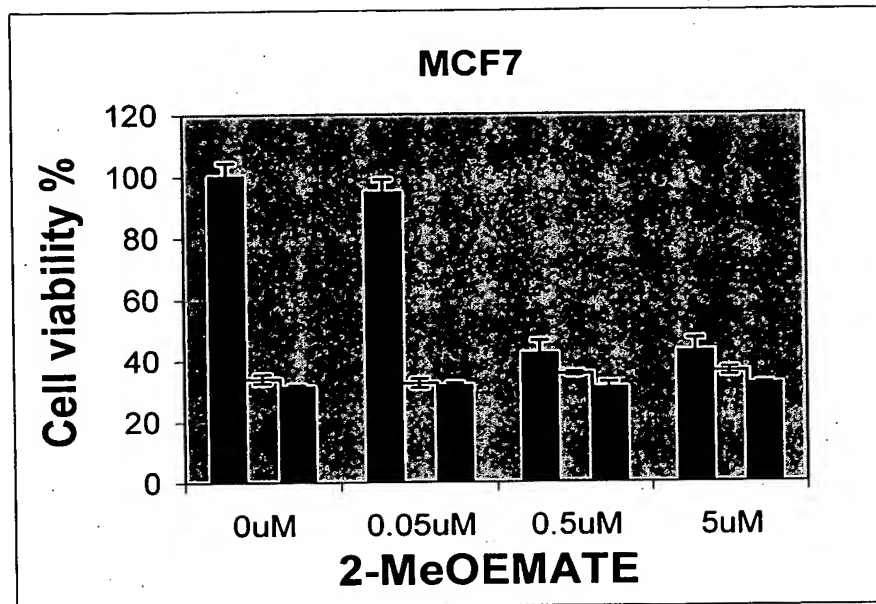


Figure 1C





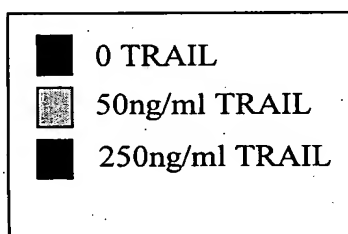
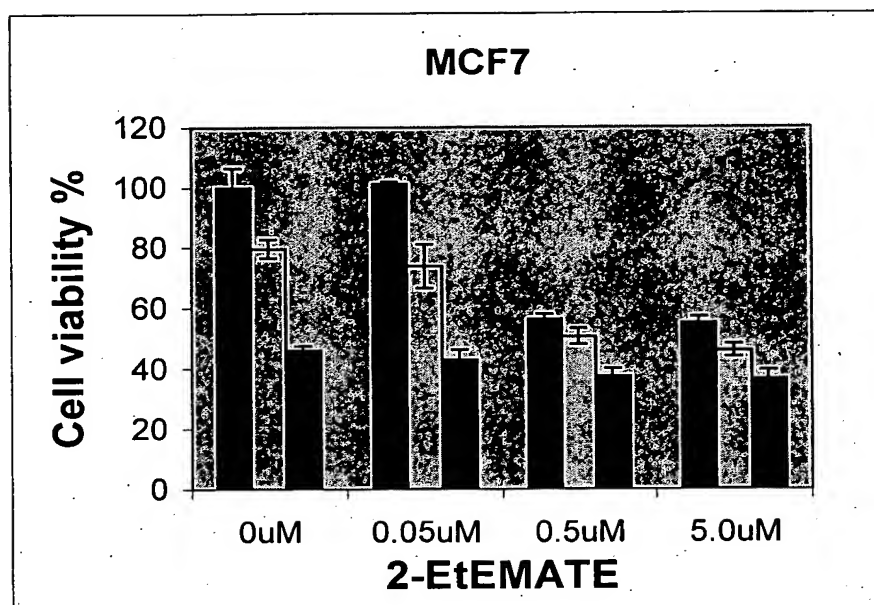


Figure 1D



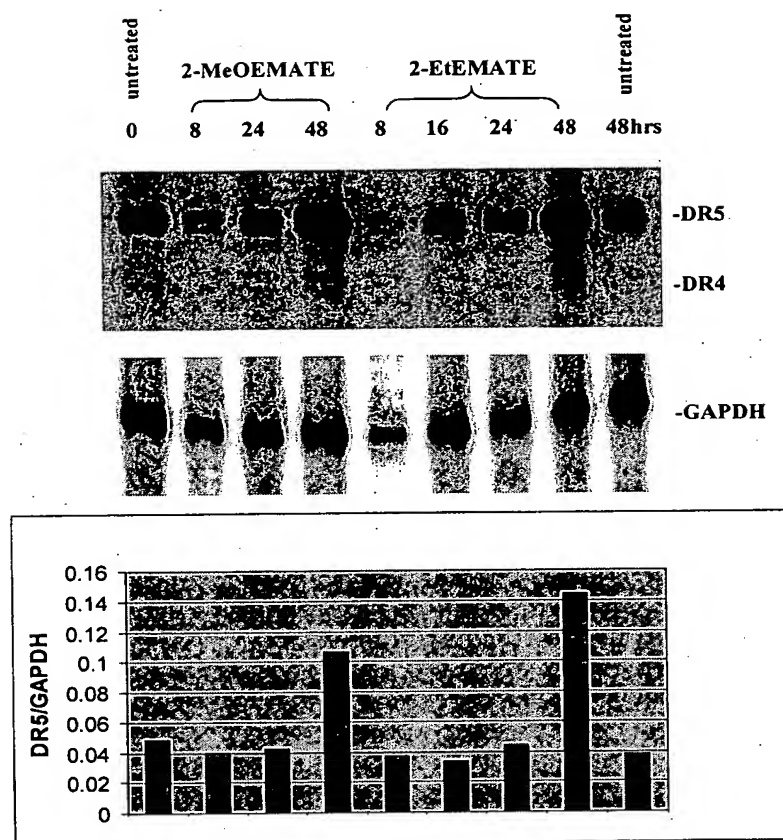


Figure 2

